Diseased fish in the freshwater trade: from retailers to private aquarists

Alberto Maceda-Veiga[1,2], Jo Cable[3]

[1]Department of Evolutionary Biology, Ecology and Environmental Sciences-Institute of Research in Biodiversity, Universitat de Barcelona (IRBio-UB), 08028 Barcelona, Spain

[2]Department of Integrative Ecology, Estación Biológica de Doñana (EBD-CSIC), Avda Américo Vespucio, s/n, 41092 Sevilla, Spain

[3]School of Biosciences, Cardiff University, CF10 3AX, UK

ABSTRACT: Millions of fish are transported between countries annually for the aquarium trade, yet no quantitative study has examined how disease frequency differs among species and stakeholders. Here we visually inspected freshwater fish species in 12 specialised and non-specialised aquarium retailers in Spain for the presence of diseased fish in 2015 and in 2016. This information was complemented with disease records from three internet fora (>100,000 users) and pathogen identification in a retailer. Overall, 22 fish species out of the 312 recorded were reported diseased, with species of Poeciliidae accounting for most records. Ich, dropsy, bacterial and monogenean infections were the most common diseases, but disease frequency differed amongst retailers and private aquarists. Although only 11 fish species in retailers were deemed unhealthy, they were the popular species amongst aquarists. We encourage improved management of fish stocks, and more education campaigns to promote fish welfare and avoid misdiagnosis in the Spanish aquarium hobby.

KEY WORDS: aquarium trade · ornamental fish welfare · pet shops · biosecurity · parasites

INTRODUCTION

The aquarium trade is popular worldwide, with a total retail value estimated at US$ 3 billion and millions of fish being transported between countries annually (FAO 2010). Stakeholders in the aquarium trade are diverse, ranging from aquarium hobbyists to general pet owners, and from specialised retailers to general pet shops (Maceda-Veiga et al. 2016). Even though fish keeping in retailers is regulated (e.g. EU's Common Entry Veterinary Document, UK's Fish Health Inspectorate), it is unknown how fish resilience to stress and disease differs amongst aquarium stakeholders even though such information is essential for developing improved management strategies.

The origins of fish diseases are multifactorial, but poor water quality and lack of quarantine procedures are two recognised causal factors in aquarium fish (Davenport, 1996; Noga 2011). The use of certificates, such as the Common Entry Veterinary Document of the European Union, should prevent the sale of diseased animals, including fish. If non-official surveys detect ill fish in the trade, this suggests the need for further training and more effective animal care schedules.

Here we inspected the Spanish aquarium trade for the presence of disease fish in 2015 and in 2016 using data from visits to specialized and non-specialized aquarium retailers, aquarists' internet fora, questionnaires and records of a disease biologist. Our specific goals were to examine whether sick fish are for sale in the aquarium trade and to identify which fish species most frequently experienced diseases at retailers and aquarists' home. We also explored whether disease frequency was associated with specific ornamental varieties and other traits related to the popularity of fish species among aquarists.

MATERIALS AND METHODS

Presence of fish with overt signs of disease in freshwater aquarium retailers

One author (A.M.V) visually inspected quarterly all fish in metropolitan based retailers in 2015 and 2016: eight in Barcelona and four in Seville; half of which were specialized and half non-specialized retailers in each Spanish province. We recorded the total number of fish species in each retailer, and the tanks which housed individuals with clinical signs of disease (e.g. white spots, clamped fins, frayed fins, dropsy, bulging eyes, underweight, external haemorrhages and ulcers; Noga 2011). An average (± S.E.) of 112±11 fish species was present in the retailers. The vast majority of tanks in retailers were well-equipped (e.g. filtration, aeration) and had between 25 and 50 of small-size individuals (< 5 cm) of each fish species. The exception was the Siamese fighting fish (*Betta splendens*), which were for sale in individual, small plastic containers without filtration.

Disease frequency was expressed as the number of visits we detected signs of disease on each fish species in each retailer divided by the total number of visits at which the species was seen in the retailer. Each retailer was the experimental unit in the statistical analyses (replicate). If the same fish species was for sale in different tanks, we calculated the disease frequency in relation to the number of tanks in which the species was present. For goldfish, which was the most frequent species in our data-set, we additionally explored whether tanks with diseased individuals were wild-type or an ornamental variety (e.g. long fins, swollen bellies). Any fish in aquaria labelled indicating quarantine were excluded from the study.

Diseased fish at aquarists' home

Three major internet fora of aquarists were checked monthly in 2015 and in 2016. On these websites, aquarists completed a questionnaire with the clinical signs of disease, often including a photograph of the fish and water quality variables. Advanced aquarists then suggested treatments and users reported the success. We used all of this information to determine the likely cause of disease from 1057 posts and data expressed as the percentage of disease cases registered. All recorded posts included a user name, date and locality to prevent a single aquarist being reported multiple times. Clinical signs and treatments provide a reasonable identification for the most common pathogens (e.g. water mold, Ich and anchor worms; Noga 2011). For instance, a fish with salt-like grains on the skin and successfully treated with malachite green was recorded as an Ich infection (*Ichthyophthirius multifiliis*). However, if the infectious agent could not be identified, we recorded the predominant overt clinical signs (behaviour alterations, cachexia, deformities, dropsy, exophthalmos and haemorrhage). For instance, a fish with largely swollen belly and scales with a pinecone-like appearance was classified as dropsy.

Aquarists' questionnaires

We complemented data from internet fora with 100 questionnaires completed by aquarists after a one-day training course on fish diseases in a retailer. The number of disease cases was expressed as percentage. Aquarists were asked to think of all diseases affecting their fish since they had started in the aquarium hobby and rank them by frequency. When pathogen identification was uncertain, we recorded the overt clinical signs (see above). Aquarists were also asked if fish mortality occurred at the beginning of setting up their aquaria, in an established aquarium (>6 months) or after the introduction of new fish.

Pathogen identification in a retailer

Pathogen identification was confirmed in one of the Spanish retailers, where any sick fish in 2015 and in 2016 was examined by a fish disease biologist. Diseased fish ($N = 212$) were placed in a Petri dish and their surface examined under a dissecting microscope. Internal gut parasites were only examined in recently, dead fish via necropsy or in alive fish via the examination of faecal material using an Olympus microscope. Pathogens were identified into broad groupings (e.g. *Saprolegnia*, Ich, *Lernaea* spp.) using rapid diagnostic techniques (e.g. smears, squash, Diff-Quick staining) following Noga (2011). Number of disease records was expressed as a percentage.

Data analyses

We top-ranked and showed number of disease records from all four information sources (retailer inspections, aquarists' fora, questionnaires and biologist) separately to identify which fish species had the highest proportion of cases registered, and to assess the suitability of these methods for monitoring aquarium fish diseases in the trade. For the retailers, we compared disease frequency among fish species and type of retailers using a generalized linear model with binomial error distribution/logit link function. Significance was assessed using the *Anova* function (the likelihood ratio χ^2 test at ≤ 0.05) within the *car* package (Fox & Weisberg, 2018) in the R software (R Core Team, 2017). Finally, we used the rank scale developed by Maceda-Veiga et al. (2013), specifically to assess whether the most popular species amongst aquarists also have the highest number of disease cases registered.

RESULTS

Our survey recorded 312 species from 14 orders and 56 families with Cichlidae (38%) and Cyprinidae (13%) being the dominant families. Most fish species on sale (97%) had a healthy appearance, but individuals of 11 species showed clinical signs of disease (Fig. 1). Amongst varieties of goldfish disease frequency was higher (73%) than that of the wild-type comet fish. Disease frequency differed amongst retailer types (χ^2=97.22; P<0.001), being 15% higher for non-specialized than for specialized retailers, but there was no significant interaction between type of retailer and fish species (χ^2=3.1; P=0.99).

The proportion of disease records from 1057 internet posts varied with fish species, being highest for *Poecilia reticulata* followed by *Xiphophorus maculatus*, *Poecilia sphenops* and *Chromobotia macracanthus* (Table 1). Results of aquarists' post were mostly consistent with those of aquarists' questionnaires, although new species (*Puntius titteya* and *Pethia conchonius*) had particularly high disease records (Table 1). Regarding when fish mortality occurred, 48 aquarists out of the 100 surveyed indicated that it was shortly after aquarium set up, 52 reported that fish died after the introduction of new fish in the tank. Species of Poeciliidae (*Xiphophorus* and *Poecilia*), *Trichogaster lalius*, *P. titteya* and *C. macracanthus* accounted for the majority of disease records from the disease biologist (Table 1).

Ich (41%), bacterial infections (12%) and dropsy (18%) accounted for the majority of records on internet fora (Fig. 2). On the questionnaires, aquarists reported that fish were only affected by Ich (62%), bacterial infections (30%) and dropsy (8%, Table 1). Out of the 212 disease outbreaks in the retailer led by the fish disease biologist, only three disease types were recorded (Ich, bacteria and monogeneans; Fig. 3). Ich infections were particularly prevalent on *C. macracanthus* and bacteria combined with monogeneans on species of Poeciliidae (*Xiphophorus* spp. and *Poecilia* spp.) (Table 1).

DISCUSSION

Our study is the first to assess disease vulnerability of freshwater species in different stakeholders of the ornamental trade, including data from aquarists' internet fora, aquarists' questionnaires, and visual surveys in specialized and non-specialized retailers.

All information sources proved to be complementary in monitoring fish diseases in the aquarium trade but, unsurprisingly, with a varying degree of accuracy. For instance, fish species vulnerability to particular diseases from aquarists' records differed from those of the retailer led by a fish disease biologist. The most plausible explanation for this difference is that general aquarists identify the most easily recognisable diseases, but pathogens such as bacteria, protists and monogeneans can, superficially, have similar symptoms (e.g. turbid and frayed fins) (Noga 2011) and without a detailed fish examination (autopsy, histopathology, microbiology and/or PCR), definitive diagnoses are not possible. We attempted to minimize misdiagnosis by interviewing aquarists who attended a training course on fish diseases, and by examining internet posts with full descriptions of fish diseases, including pictures, successful treatments and the water quality of aquaria. Nevertheless, misdiagnosis most likely explains why monogenean infections on Poeciliidae were detected by the fish disease biologist but not by aquarists at home. Regardless of the expertise in disease diagnosis, changes in the environment, diet, chemical treatments, and cumulative stress due to handling and transport from retailers to home also affect fish vulnerability to disease (Davenport, 1996; Sobhana et al. 2002; Noga 2011). Therefore, our results may be due to differences in fish species sensitivity to poor water quality rather than to differences in their vulnerability to pathogens *per se*. Nonetheless, the fact that some fish species had high disease frequencies suggests that their management should be improved.

Overall, guppies (*P. reticulata*), mollies (e.g. *P. sphenops*), platies (e.g. *X. maculatus*) and swordtails (*X. helleri*), all Poeciliidae, were popular aquarium species with particular high number of disease records, probably because selective breeding often results in inbreeding, which is a major risk factor of disease (e.g. Langen et al. 2011; Smallbone et al. 2016). In our study, this hypothesis was confirmed in *C. auratus* because its varieties had higher disease records than the wild-type. Breeding for non-health related traits (e.g. appearance) may have led to inadvertent selection for decreased disease resistance (Ballou 1993; Spielman et al. 2004; Smallbone et al. 2016). Poeciliid fish and goldfish are in the top 30 most frequent aquarium fish species around the world (Strecker et al. 2011; Maceda-Veiga et al. 2016), probably because aquarists like fancy breeds, their low price and many magazines and retailers recommend these 'hardy' species for beginners (pers. observ.). Poeciliid fish and goldfish varieties were probably hardy fish decades ago but have become highly susceptible to acquire diseases due to the loss of allelic diversity, in particular heterozygosity in the Major Histocompatibility Complex (Schenekar & Weiss 2017). Therefore, it is necessary to revise the genetic quality of these varieties. Moreover, high fish mortality shortly after the aquarium set up suggests that retailers should enforce education campaigns to beginners.

Our study showed that Ich, bacterial and monogenean infections had the highest number of disease cases in the aquarium trade (Fig. 2). This was expected because generalist pathogens with direct, fast life-cycles are amongst the most common diseases in aquaculture (Davenport 1996; Noga 2011; Austin et al. 2012). The rapid cycle of these pathogens and fast turnover of fish stocks also reduced the risk of recounting the same diseased individuals in our quarterly visits to each retailer year around. Although there was high variability in fish species vulnerability to disease, *C. macracanthus* had a particularly high frequency of Ich outbreaks and *P. titteya* and *P. conchonius* seemed to be particularly prone to dropsy. Since fish scales are a barrier against disease (Rottmann et al. 1992), the lack of scales in *C. macracanthus* might explain Ich outbreaks. However, we did not detect Ich outbreaks in other popular scale-less fish hosts (e.g. *Pangio kuhlii*). Water quality might have been a confounding factor for these fish species because even small changes in water quality parameters might alter infection dynamics (e.g. Hoole et al. 2008; Noga 2011). Poor environment is likely to be a major causal factor for diseased *B. splendens* in small pots in retailers, which also may be the reason why this species often displays signs of disease in home aquaria. For dropsy, we found some aquarists reporting success with nifurpirinol baths, suggesting a bacterial origin (Noga 2011). However, dropsy is a multifactorial disease, which may have a non-infectious origin, including physiological dysfunctions (Noga 2011). Besides fish traits and environmental conditions, the disease risk of fish may be due to poor diet because most aquarists fed fish exclusively with standard flakes.

Despite the sale of sick animals being prohibited in the pet trade, we did find ill fish in the licensed Spanish aquarium trade; an issue that particularly affects 11 species frequently found in retailers. We encourage improved management of aquarium fish, particularly poecillid and goldfish stocks, and more education campaigns to promote fish welfare and avoid misdiagnosis in the Spanish aquarium trade.

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Table 1 The 15 aquarium fish species with the highest number of disease cases registered based on aquarists' questionnaires, internet fora and the records of a disease biologist in a retailer. In bold the fish species listed in the top 20 most frequent fish species in the aquarium trade *sensu* Maceda-Veiga et al. (2013).

Aquarists' questionnaires[1]	%	Aquarists' internet fora[1]	%	Disease biologist[2]	%
Xiphophorus maculatus	30	***Poecilia reticulata***	15	***Poecilia reticulata***	16
Poecilia reticulata	25	***Xiphophorus maculatus***	13	*Poecilia latipinna*	15
Carassius auratus	13	***Poecilia sphenops***	12	***Xiphophorus hellerii***	15
Puntius titteya	9	*Chromobotia macracanthus*	8	***Xiphophorus maculatus***	11
Xiphophorus hellerii	8	***Betta splendens***	8	***Carassius auratus***	9
Chromobotia macracanthus	5	***Carassius auratus***	8	***Paracheirodon innesi***	9
Xiphophorus variatus	4	***Pterophyllum scalare***	7	*Trichogaster lalius*	8
Poecilia sphenops	3	*Symphysodon discus*	5	*Chromobotia macracanthus*	3
Paracheirodon innesi	1	***Xiphophorus variatus***	5	***Poecilia sphenops***	2
Pethia conchonius	1	***Corydoras aeneus***	4	*Poecilia vellifera*	2
Pterophyllum scalare	1	***Hypostomus plecostomus***	4	*Puntius titteya*	1
Others	<1	*Trichogaster lalius*	4	*Paracheirodon axelrodi*	1
		Paracheirodon axelrodi	2	*Gnathonemus petersii*	1
		Paracheirodon innesi	1	***Trigonostigma heteromorpha***	1
		Carinotetraodon travancoricus	1	*Micropoecilia wingei*	1
		Others	3	Others	5

[1]Aquarists declared that all fish were vulnerable to Ich and bacterial infections but that *C. macracanthus* was highly prone to Ich and that *P. titteya* and *P. conchonius* were to dropsy

[2]All fish species were vulnerable to bacterial and Ich infections. However, *C. auratus* and Poeciliidae (*Xiphophorus* and *Poecilia*) were also highly prone to monogenean infections (>56% fish inspected had worms), and *C. macracanthus* was highly prone to Ich (Ich cause >90% of disease reports)

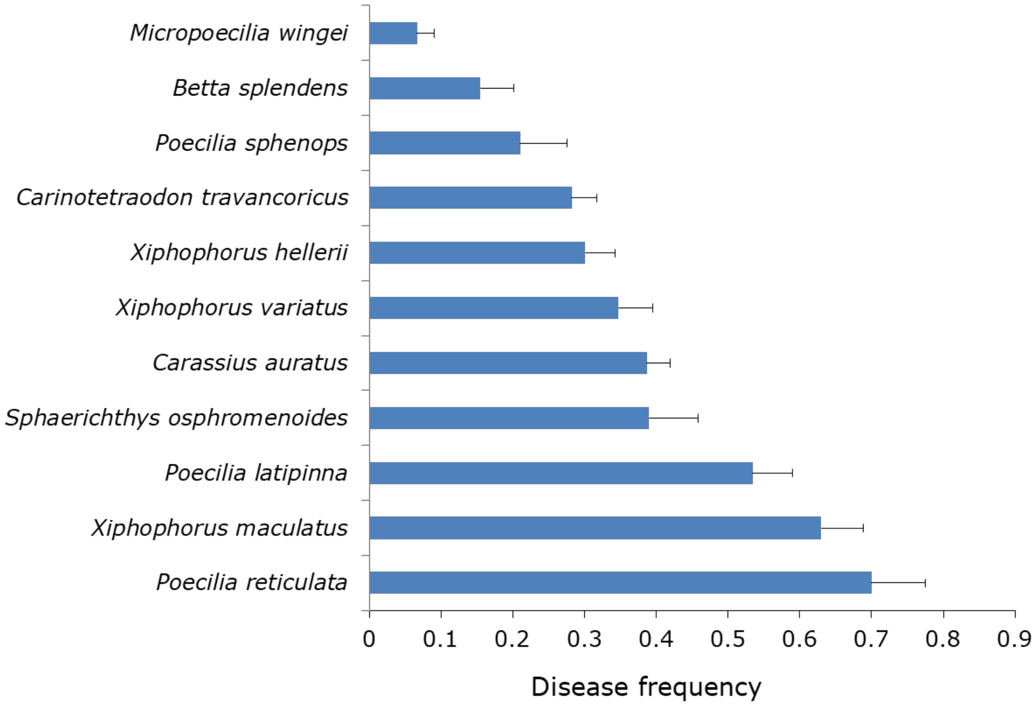

Fig. 1. Changes in the mean disease frequency (± S.E.) of the 11 fish species found diseased in the 12 Spanish aquarium retailers (e.g. we detected ill fish in about 70% of 12 checks on *Poecilia reticulata* stocks). Only these 11 species had signs of disease out of the 312 species present and all 11 fish species were offered for sale in the 12 retailers.

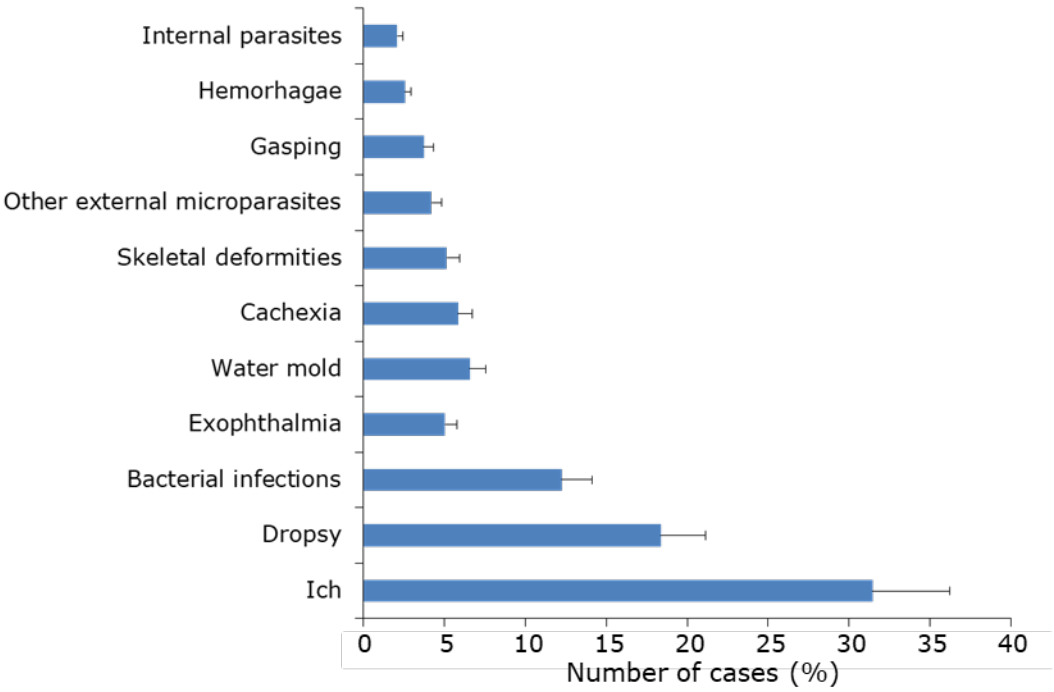

Fig. 2. Changes in the mean percentage of signs of disease (± S.E.) in home aquaria based on aquarists' internet fora (e.g. Ich outbreak found in about 30% of the 1057 posts examined).

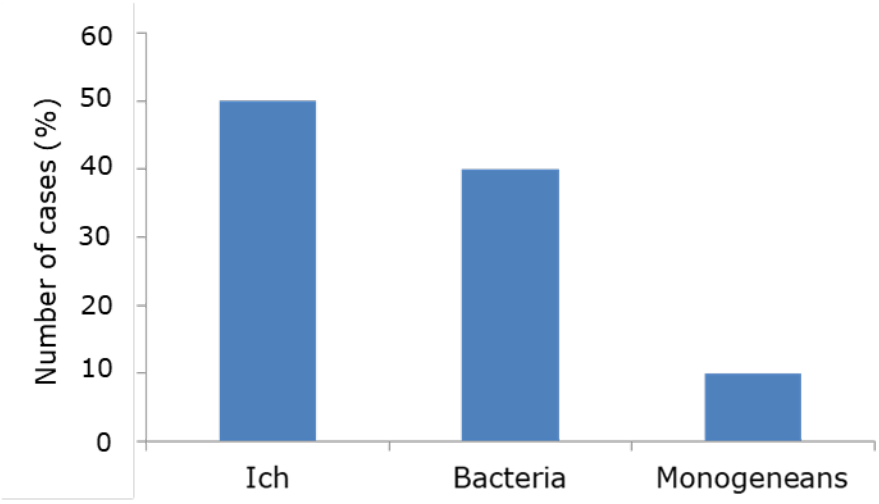

Fig. 3. Changes in the number of cases of the three most common diseases (Ich, bacteria and monogenean infections) using the records of a fish disease biologist in a retailer.